

## Paraphenylenediamine, a new color test for lichens

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Dr. Alexander W. Evans, of the Osborn Botanical Laboratory at Yale University, has informed me of a new color test for the genus *Cladonia*, which promises an interesting re-survey of the reactions of its many species. He learned of it from Dr. Heinrich Sandstede, of Germany, who reports that it was discovered by a Japanese botanist, Asahina. The reagent is called Paraphenylenediamine, or Paradiaminobenzine.

Hitherto a solution of KOH, potassium hydroxide or caustic potash, has been the reagent for determining the presence of relatively large amounts of the bitter tasting fumarprotocetraric acid in lichens, and has been satisfactory in giving color reactions in many species, which are helpful in separating them from other species of somewhat similar appearance to the unaided eye or the hand lens. These reactions are described in Lindau's "Die Flechten für Anfänger," which a number of members of the Torrey Botanical Club are now using for its excellent plates; in Annie Lorain Smith's "Manual of British Lichens," and in Dr. Evans "Report on the Cladoniae of Connecticut," and I have followed these authorities in citing such reactions in papers on the Cladoniae of our range and of the North Woods, published in Torreya.

But German botanists, especially Dr. Sandstede, have named a number of species and forms on the basis of their taste to the human tongue, whether bitter because of a relatively large amount of fumarprotocetraric acid, or mild because without it. Now, with the use of paraphenylenediamine, says Dr. Evans, "it will no longer be necessary to taste our specimens." His letter continues:

"This is a crystalline substance, soluble in alcohol, and is applied by means of a medicine dropper to the dry plant. If fumarprotocetraric acid is present a yellow color quickly appears and the color deepens to an orange, orange red, or brick red as the alcohol evaporates. If the bitter acid is not present, the reaction is wholly negative or a pale and permanent yellow stain results. Dr. Sandstede recommends that a small amount of the reagent be dissolved in a watch-glass and tested in such a very bitter species as *C. coniocraea* to see if the solution is

strong enough. If it gives a reaction with this, it is all right. The solution must be freshly prepared, since it rapidly deteriorates, but the same solution can be used for many tests. If the bitter *C. tenuis* and the mild *C. mitis* are placed side by side and tested, the contrast between the two is brought out very vividly. I have applied the reagent to the various specimens referred to *C. Grayi* and have obtained a negative result every time."

Dr. Evans' reference to *C. Grayi* is interesting, for it concerns forms of cupped Cladoniae, which, to the naked eye and hand lens appear much the same. In my papers, I used *C. chlorophaea*, for the species, with the forms described by Dr. Evans in his Connecticut Report. But in additional notes on the Connecticut species, in Rhodora, as cited in my papers, Dr. Evans notes that Dr. Sandstede makes some of these cupped forms *C. Grayi*, basing them on material sent to him, from North Carolina, by Rev. Fred. W. Gray, of Philippi, West Va., for whom Dr. Sandstede named the species. *C. Grayi*, according to Sandstede, is mild to the taste, and he names it as a separate species for that reason but no morphological distinction is described. Similar cupped Cladoniae, with brown apothecia when fertile, in the Group Thallostelides, which are bitter, may presumably be left in *C. chlorophaea*, although I have not obtained definite advice on that yet from Dr. Evans. *C. Grayi* may be simple, outwardly resembling what I have called, following Dr. Evans' report, *C. chlorophaea*, *f. simplex*, or with branches from the rims bearing brown apothecia, like *C. chlorophaea*, *f. carpophora*.

I have obtained some of the paraphenylenediamine and am trying it out, with surprising results, which will lead to a complete re-test of all the Cladonia species and forms in my collections to see what happens to them under this new and powerful reagent. On *C. coniocraea*, which gives a brown reaction, sometimes but slowly, with KOH, the yellow, orange red and brick red colors appear, as Dr. Evans says. I got a brick red reaction in *C. verticillata*, which gives no or only a slight brown reaction with KOH, and the same happened with *C. calycantha*. *C. santensis*, which reacts yellow with KOH, does the same with P— (we will have to find a shorter name for it.) I tried some of the cupped Cladoniae and in some got no reaction, and in others a

brick red; presumably the first are *C. Grayi* and the second *C. chlorophaea*.

As Dr. Evans suggests, the test will be interesting as between species of the subgenus *Cladina*, the "Reindeer Mosses," some of which look much alike to the naked eye. I have just begun the retesting of my attic full of collections, but I hasten to inform other members of the Torrey Botanical club of this new reagent so that they may have the pleasure of trying it as well. Future lichen floras will have to include references to the test with paraphenylenediamine as well as KOH.

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